Removal of Selenium from River Water in Organic Carbon Coated Sand Columns by *Enterobacter taylorae* Isolated from a Rice Straw Bioreactor

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PROJECT SUMMARY

Discovering an effective means to remove selenium (Se) from Se-contaminated water is fundamental in minimizing environmental contamination and ensuring wildlife protection. In this study, *Enterobacter taylorae* attached to tryptic soy agar (TSA) coatings was used in sand columns to remove Se from a natural river water. During 80 days of the experiment, *E. taylorae* and indigenous Se(VI) reducers in the water used organic carbon coatings to effectively reduce Se(VI) to Se(0). About 95 and 70% of the influent Se (469 µg/L) was reduced to Se(0), respectively in the columns with and without *E. taylorae* and 94-98% of the newly-formed Se(0) was trapped in the columns. Analysis of Se species in effluent at the end of the experiment revealed that organic Se from the columns with *E. taylorae* was similar to that in influent (0.567 µg/L). This study indicates that using organic coatings attached with Se(VI) reducers in a biotreatment system may be a potentially feasible method to remove Se from Se-contaminated water in field.

INTRODUCTION

Elevated selenium (Se) of agricultural drainage water had created serious hazards to fish and waterfowl in the San Joaquin Valley, California (Ohlendorf, 1989; Presser and Ohlendorf, 1987). Concerns for the safety of these waterfowl production areas make it very important for scientists and wetland managers to find ways for removing Se from agricultural drainage water before it is disposed into aquatic systems.

Reduction of Se(VI) to Se(0) is one of the major biogeochemical processes in aquatic systems (Gao et al., 2000; Velinsky and Cutter, 1991; Weres et al., 1989; Zhang and Moore, 1996). Many bacteria isolated from aquatic systems have been found to be capable of reducing Se(VI) to Se(0), i.e. *Wolinella succinogenes*, *Pseudomonas stutzeri*, *Sulfurodpirillum barnesiii*, *Enterobacter cloacae*, *Thauera selenatis*, *Enterobacter taylorae*, and *Citrobacter freundii* (Francisco et al., 1992; Lortie et al., 1992; Cantafio et al., 1996; Losi and Frankenberger, 1997; Oremland et al., 1999; Zahir et al., 2003; Zhang et al., 2004a). The reduction rates of Se(VI) are affected by microbial activity, competitive electron acceptors, effective organic carbon sources as energy/electron donors, and various environmental conditions (Francisco et al., 1992; Lortie et al., 1992; Cantafio et al., 1996; Losi and Frankenberger, 1997; Oremland et al., 1999; Zahir et al., 2003; Zhang et al., 2003; 2004a). Because of the insolubility of Se(0) in aquatic systems, reduction of Se(VI) to Se(0) is considered to be a useful technique for removing Se from Se-contaminated water.

A general bacterial treatment system for removal of Se from Se-contaminated water often uses a liquid phase of organic carbon sources such as acetate, lactate, and glucose (Cantafio et al., 1996; Losi and Frankenberger, 1997; Oremland et al., 1999; Zahir et al., 2003; Zhang et al., 2003; 2004a). Only small amounts of the liquid phase of organic carbon are commonly used by Se(VI) reducers to reduce Se(VI) before it flows out of the system with the treated water (Tucker et al., 1998). A large amount of organic carbon often goes waste due to inefficient usage, which results in a high cost of these expensive chemicals for biotreatment in the field. Therefore, if the organic carbon is fixed in a biotreatment system as a solid particle on which Se(VI) reducers can attach and use it as a source of energy and electron donors to reduce Se(VI) to Se(0) when soluble Se(VI) passes through, the loss of organic carbon by flowing water would be limited, which would potentially reduce the cost for treating Se-contaminated water.

In this study, we tested the use of an organic carbon-coated sand column for removal of Se from Se-contaminated water by a Se(VI)-reducing bacterium, *Enterobacter taylorae*.

MATERIALS AND METHODS

River water

River water used in this study was collected from New River, California. The water, with a pH of 8.2 and salinity [electrical conductivity (EC)] of 2.3 dS/m, contained 5 µg/L Se(VI), 1 µg/L of selenite [Se(IV)], 0.567 µg/L of organic Se, 9.23 mg/L of NO_3^--N, 0.04 mg/L of NH_4^+-N and 0.88 mg/L of PO_4^{3-}-P. The river water was passed through a 5 µm filter to remove detritus prior to use. Se standard solution [Se(VI), 10,000 mg/L] was passed through a sterile 0.2 µm membrane filter prior to its addition to the water. The final Se concentration in the water (influent) was 469 µg/L.

Tryptic soy agar-coated sand column

In one of our previous studies, we had isolated a Se(VI)-reducing bacterium, *E. taylorae* from a rice straw bioreactor and used this bacterium to reduce Se(VI) in artificial and natural drainage water in the laboratory batch studies (Zahir et al., 2003; Zhang et al., 2003). During the isolation, we found that the bacterium colonies can tightly attach to the surface of tryptic soy agar (TSA) (DIFCO, Becton Dickinson, MD) and rapidly reduce Se(VI) to red Se(0). Slow-moving deionized water did not wash away these colonies. Inspired by these observations, we fixed tryptic soy agar (an organic carbon source) on the surface of sand in this study and then used the organic coatings as sites for *E. taylorae* to attach and use this fixed carbon source to reduce Se(VI) to Se(0). Sand and 5% of the TSA solution with 0.05% of each glucose and yeast extract (DIFCO, Becton Dickinson, MD) were separately autoclaved (18 psi at 121 °C) for 20 minutes. Hot sand was moistened with the hot TSA solution, and then spread onto a sterile plate until cooling to room temperature (21 ºC). Aggregates of the TSA-coated sand were separated by hand.

Flow-through bioreactor

The flow-through bioreactor consisted of three units: a flask containing the river water spiked with Se(VI) to a final Se concentration of 469 µg/L, a peristaltic pump, and 60-ml syringes used as TSA-coated sand columns. The columns were filled with sterile glass wool at the bottom, TSA-coated sand, and sterile glass beads on top. After the addition of a washed *E. taylorae* cell suspension, the columns were incubated for 3 days, and then the river water (without adding any additional organic carbon sources) was pumped through the columns with a hydraulic residence time of ~0.7 day. The experiment was run in duplicate at a room temperature (21 ºC). The columns without the addition of *E. taylorae* served as a control. Effluent water samples were collected twice a week for the first 54 days and then weekly for the rest of the experiment. The water samples for total Se and total soluble Se were stored in a freezer until analysis. Se species in water samples were analyzed at the end of the experiment.

Analysis

Total Se, total soluble Se, and Se species [Se(VI), Se(IV) and organic Se] in the water samples were determined by a method developed by Zhang and Frankenberger (2003a). Se concentrations in prepared solutions were analyzed by hydride generation atomic absorption spectrometry (HGAAS) (Zhang et al., 1999). The methods for calculation of Se(0) and Se mass in the biotreatment system are presented in Table 1.

RESULTS AND DISCUSSION

Changes in concentrations of Se in the influent and effluent from the TSA-coated columns are illustrated in Fig. 1. During 80 days of the experiment, total Se in the influent had little change, ranging from 464-473 µg/L. However, total Se and total soluble Se were dramatically altered in the effluent when Se passed through the TSA-coated columns. In the columns with *E. taylorae*, total Se and total soluble Se dropped rapidly to a level of 15.3-20 µg/L in the first 20 days of the experiment, and remained at this level to day 52. Total Se and total soluble decreased to a low level of 3.45-6.54 µg/L during the rest of the experiment. Rapid removal of the added Se(VI) in the TSA-coated sand columns was attributed to bacterial reduction of Se(VI) to Se(0), which was visible by the observation of red Se(0) precipitates on the surface of the coatings at the lower part of the columns after the experiment. The Se(VI) reducing bacterium, *E. taylorae* was isolated from a rice straw bioreactor for removal of Se(VI) from drainage water (Zhang and Frankenberger, 2003b). It has been used to effectively reduce 95% of the added Se(VI) (1000 µg/L) to Se(0) in a medium consisting of 500 mg/L of yeast extract during a 7-day batch experiment and directly reduce Se(VI) in natural drainage water collected from the western San Joaquin Valley, California (Zhang et al., 2003). This study reveals that *E. taylorae* can also effectively reduce a large amount of Se(VI) to Se(0) in a flow-through experiment. When a Se(VI)-reducing environment was optimized in the columns, reduction of Se(VI) to Se(0) occurred more effectively. In the final several days of the experiment, about 99% of added Se(VI) was reduced to Se(0) in the columns with *E. taylorae* added.

Not all of the reduction of Se(VI) to Se(0) was caused by *E. taylorae*. In the control columns without the addition of *E. taylorae*, total Se and total soluble Se also dropped to a range of 40.1-78.8 µg/L during the first 34 days and slowly decreased to 10.4-36 µg/L at the end of the experiment (Fig.1). In a batch study on the reduction of Se(VI) to Se(0) in the same New River water by *Citerobacter freundii*, Zhang et al. (Zhang et al., 2004a) reported that Se(VI) concentration slightly changed in the non-sterile river water without the addition of *C. freundii* during the first 5 days of the experiment, and then decreased rapidly from 968 to 168 µg/L, revealing that the existence of indigenous Se(VI) reducers in this river water that can contribute to Se(VI) reduction after acclimation. In this study, the existence of unknown indigenous Se(VI) reducers in the water resulted in reduction of Se(VI) to Se(0) in the control columns. However, the addition of *E. taylorae* into the columns significantly enhanced the reduction of Se(VI) to Se(0), with a 94.6-95% reduction of the added Se(VI) to Se(0) in the columns with *E. taylorae* added and a 70-70.2% reduction in the columns without the addition of *E. taylorae*.

Effective trapping of the newly-formed Se(0) in a biotreatment system is important to determine the effectiveness in bioremediation of Se-contaminated water (Barton et al., 1994; Zhang and Frankenberger, 2003b; Zhang et al., 2004b). In a recent study on the fate of newly-formed Se(0) in aquatic systems, Zhang et al. (2004b) reported that newly-formed Se(0) can be easily oxidized to Se(IV), and further to Se(VI) if newly-formed Se(0) flows out of a treatment system to a nearby aquatic system. Therefore, newly-formed Se(0) must be trapped before it flows out of a biotreatment system with treated water. This study reveals that the newly-formed Se(0) was effectively trapped in the columns. Calculation of Se mass in the influent and effluent indicated that 95 and 70% of the added Se(VI) in the water was reduced to Se(0) when it passed through the columns with and without *E. taylorae*, respectively, and 94-98% of the newly-formed Se(0) was trapped in the columns (Table 2).

Fixation of organic carbon on the surface of sand particles as solid organic coatings can significantly reduce the cost for the removal of Se from Se-contaminated water because large amounts of the fixed organic carbon would be efficiently used by Se(VI)-reducing bacteria to reduce Se(VI) instead of flowing out of the system with treated water. In this study, only very small volumes of the TSA solution were used to moisten sand. During 80 days of the experiment, we did not add any additional organic carbon sources to the columns and influent. *E. taylorae* and other unknown indigenous Se(VI) reducers in the New River water used TSA coatings to effectively reduce Se(VI) to Se(0), with a 93% removal of the added Se(VI) in the columns inoculated with *E. taylorae*.

Concerns on bioavailability of Se in treated water have increased recently since the discovery of much higher bioavailability of Se in treated water than the influent in an algal-bacterial treatment system (Amweg et al., 2003). The bioavailability of Se in aquatic systems is largely dependent on the speciation of Se present (Besser et al., 1993; Lemly et al., 1993; Wang and Lovell, 1997). Studies by Besser et al. (1993) and Wang and Lovell (1997) showed that organic forms of Se have higher bioavailability than

Se(IV)] or/and Se(VI), and bioaccumulate more rapidly. Increases in concentration of the most bioavailable organic Se in treated water creates greater problems to biota than that in influent (drainage water) (Amweg et al., 2003). Production of soluble organic Se may be related to the amounts of soluble organic materials used by bacteria to reduce Se(VI) to Se(0) in biotreatment systems. Although we do not know the total amounts of organic Se produced during this experiment because we did not monitor the changes of organic Se in the effluent throughout the study, analysis of Se species on the final day of the experiment shown that organic Se was 0.44-0.61 µg/L in the columns inoculated with *E. taylorae*, which was very close to the concentration of organic Se in the influent river water. Organic Se was relatively higher (2.25-2.95 µg/L) in the effluent from the columns without *E. taylorae*.

Agricultural activity in California generates high-Se drainage water (Sylvester, 1990; Setmire and Schroeder, 1998). Se needs to be removed before its disposal to the nearby wetlands. This study shows that Se(VI) reducers attached to organic coatings can effectively reduce soluble Se(VI) to Se(0) when it passed through sand columns and it may be a potentially feasible method to remove Se from agricultural drainage water.

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Table 1. Equations for calculating Se mass in the columns.

Se	Equations
Total input Se to the columns	$\sum_t$ (water flow rate * Se in influent)
Total soluble Se output from the columns	$\sum_t$ (water flow rate * soluble Se in effluent)
Total Se(0) output from the columns	$\sum_t$ (water flow rate * Se(0) in effluent)
Total trapped Se(0) in the columns	$\sum_t$ (total Se in influent - total Se in effluent in each column)

The term t is time.

Table 2. Mass of Se (µg) in the TSA-coated sand treatment system.

Columns	Total input Se	Trapped Se(0)	Soluble Se output	Se(0) output	Trapped Se(0) /Total input Se
1	1501	1002	440.7	58.3	66.8
2	1501	998	447.1	55.9	66.5
3	1501	1388	81.8	31.2	92.5
4	1501	1402	75.1	23.9	93.4

Table 3. Soluble Se species in the effluent on the final day of the experiment

Columns	Se(VI)	Se(IV)	Organic-bound Se	Total Se
1	4.05	3.38	2.92	10.35
2	4.05	25.7	2.25	32
3	1.58	1.43	0.44	3.45
4	1.65	4.04	0.61	6.3

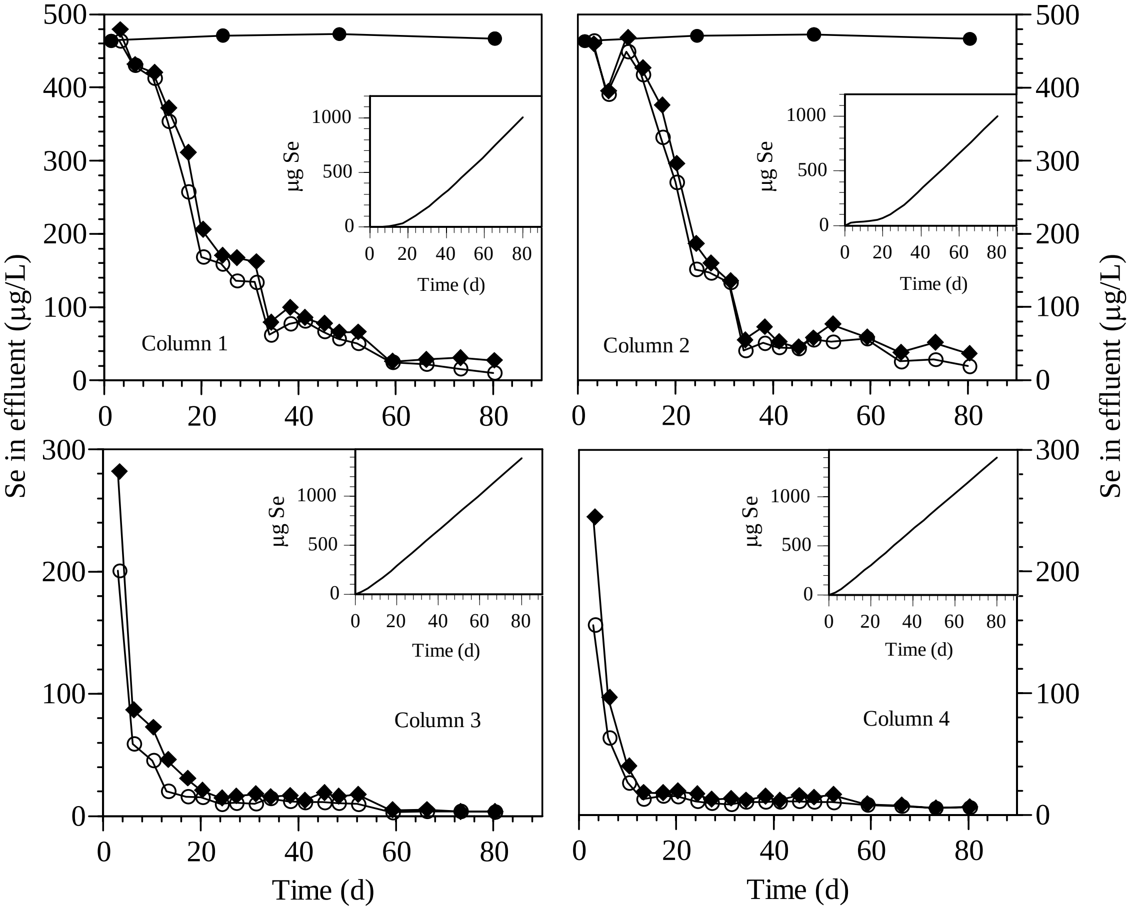

Fig. 1. Removal of Se from New River water with (duplicated columns 3 and 4) and without (duplicated columns 1 and 2) *Enterobacter taylorae* in the tryptic soy agar-coated sand columns. ◆: Total Se; ○: Total soluble Se; and ●: Total Se in influent. Inserted small figures show cumulative elemental Se in each column.